

## **REMARKS**

### **Regarding the Response**

Claim 1 has been amended as set forth in the above Complete Listing of the Claims. As amended, the claims are supported by the specification and the original claims. Specifically, claim 1 has been amended to clarify the structure of the claimed TRPs. Support for such amendment is provided in the specification at pages 19, ll. 15-18 and p. 20, ll. 21-25.

New claim 29 has been added. Support for claim 29 is provided in the specification as filed, specifically at page 15, Fig. 6 and in Table 1, pages 21-22.

No new matter has been added, as defined by 35 U.S.C. § 132.

In view of the finality of the April 10, 2008 Office Action and to ensure substantive consideration of this response, a Request for Continued Examination is concurrently submitted herewith, together with payment of the appertaining RCE fees (see *infra*, "Fees payable").

Thus, upon entry of the Response, claims 1, 2, 5-20, and 23-28 will be pending, of which claims 10-17 are withdrawn.

### **Rejection of Claims 1-9 and 18-28 Under 35 U.S.C. §103**

In the Final Office Action mailed April 10, 2008, the examiner has rejected claims 1-9 and 18-28 under 35 U.S.C. § 103(a) as unpatentable over Published U.S. Patent Application No. 2004/0197867 (hereinafter "the '867 publication"), further in view of U.S. Patent No. 5,013,649 (hereinafter "the '649 patent") and Leighton, M., et al., J. Biol. Chem., (2003) p. 18478-18484, vol. 278, n. 20 (hereinafter "Leighton et al.").

In the Final Office Action at page 3, the examiner has stated that applicants' previous arguments were not persuasive. In response, applicants provide the following.

On page 3 of the Final Office Action, the examiner states that "the '649 Patent and Leighton reference had already disclosed that it was known in the art that the 'furin activation domain' (FAD), comprising an amino acid sequence of the instantly-elected SEQ ID NO: 14, fused with the 'tissue regeneration domain' (TRD), comprising an amino acid sequence of the instantly-elected SEQ ID NO: 1, is the equivalent of the hBMP proprotein." The examiner also states that

the '867 publication discloses a structure of PTD-BMP2 peptide (Final Office Action, p. 4), and that in view of the combination of the cited references, the present application is rendered obvious. Applicants respectfully disagree.

It is elemental law that in order for an invention to be obvious, the difference between the subject matter of the application and the prior art must be such that the subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the art. In order to meet this standard for a proper §103 rejection, all claim limitations must be disclosed or derivable from the cited combination of references, there must be a logical reason to combine the cited references to produce an operable combination and there must be a reasonable expectation of success (*see* MPEP §2143).

The claims of the application that are pending and under examination recite a polypeptide (claims 1, 2, 5-9) and compositions (claims 18-20 and 23-28). All of the composition claims depend directly or indirectly on the polypeptide of claim 1. Therefore all pending claims of the present application recite a polypeptide (TRP) that comprises a PTD, a FAD and a TRD. Claim 1 has also been amended to include a recitation of the structure as having a linear structure.

However, the claims are not simply structural recitations, but also comprise functional description, where the TRP is activated by cleavage of the FAD and which stimulates the growth or formation of tissues or to induce the regeneration of tissues. Therefore structural recitations that are similar to those of the claims, but do not possess the claimed activity are not encompassed by the claims. Similarly, cited references showing a similar structure must also possess the recited function in order to render the claimed invention obvious.

It is well known that the biological characteristics of a protein are determined by the secondary and tertiary structure of the protein, rather than the primary structure of the amino acid sequence.<sup>1</sup> Secondary structure of the protein is determined by  $\alpha$ -helix and  $\beta$ -sheet structures of the polypeptide chain<sup>2</sup> and the  $\alpha$ -helix and  $\beta$ -sheet structures are determined by physical and chemical characteristics of the surface of protein, such as hydrogen bond(s), disulfide bond(s), hydrophobic interaction(s) and so on.<sup>3</sup> Additionally, a protein has a quaternary structure based

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<sup>1</sup> Branden, C., Tooze, J., Introduction to Protein Structure, 2d Ed., Garland Publishing, Inc., New York, N.Y. (1999); p. 4-12. (Exhibit A)

<sup>2</sup> *Id.*, p. 13-19.

<sup>3</sup> *Id.*

on physical and chemical characteristics of loops, helix and sheet structure.<sup>4</sup> Slight changes in pH or temperature can convert a solution of biologically active protein molecules in their native state to a biologically inactive denatured state and proteins in the native state are highly ordered in one main conformation whereas the denatured state is highly disordered, with the protein molecules in many different conformations.<sup>5</sup> Therefore it is well known that proteins with the same primary structure may have completely different secondary, tertiary and/or quaternary structures.<sup>6</sup>

Structurally, the TRPs of the invention “have no three-dimensional stereoregularity...in common [with] previously known active BMPs.” (Specification, p. 7, ll. 13-15.) Claim 1 has been amended to clarify that the claimed TRPs have a linear structure and do not require the 3D structure of the prior BMPs.

It is shown in the present application that creation of the recited TRPs with the linear conformation provided a needed benefit over the prior art. As repeatedly stated in the application, prior art BMPs were known in a biochemically active state with the 3D structures shown in Figs. 1 and 2 and therefore difficult to separate, purify, store, handle and administer, due to the activity and conformation. (Specification, p. 3-4.)

At pages 21-22 the specification provides Table 1, illustrating the differences between the claimed TRPs and the proteins previously known:

Table 1

Items	Prior bone morphogenetic proteins (rhBMP-2 or rhBMP7)	Inventive fusion polypeptides (TRPs)
Production method	Culture transformed CHO cells	Culture transformed <i>E. coli</i>
	Produce while maintaining transformed CHO cells	Maintain transformed <i>E. coli</i> in a simple manner
	Separate from large cell medium, and concentrate	Separate directly from <i>E. coli</i> , and dilute
Production cost	Very high	Significantly lower than those of the prior art
Production equipment and facilities	Require large-scale equipment and facilities	Very simple
Products	Activated rhBMP2, rhBMP7	Fusion polypeptides of PTD-FAD-TRD
Three-dimensional structure	Active three-dimensional structure (FIG. 1 and FIG. 2)	Non-activated peptides
	Naturally occurring structure	Naturally non-occurring random structure
Solubility in cleaning material and physiological saline	Soluble; Loss of three-dimensional structure and activity	Insoluble; No effect on structure and activity

<sup>4</sup> *Id.*

<sup>5</sup> *Id.*, p. 90.

<sup>6</sup> *Id.*, p. 90-95; See also world wide web address page lsbu.ac.uk/water/protein2.html (Exhibit B).

Items	Prior bone morphogenetic proteins (rhBMP-2 or rhBMP7)	Inventive fusion polypeptides (TRPs)
Need of carrier for administration in vivo	Diffuse rapidly due to water-solubility. Need suitable carrier for local administration	Are insoluble and transduced rapidly into adjacent cells. No need carrier for local administration
Stability of storage	Loss of activity upon breakdown of three-dimensional structure; Not storable above 37°; low stability of storage	Independent of structure; Storable above 37°; Good stability of storage
Biological mechanism	Directly bind to cell membrane receptors (direct action)	Activated proteins are secreted out of cells (indirect action)
Intracellular specific structure binding to protein after administration	No relevant data	F-actin
Intracellular processing	No relevant data	Activation by cleavage with furin enzyme
Temperature dependency in administration	Needs live body temperature to be bound to receptors	Permeate all living cells independently of temperature
Half time for activation	No relevant data	3-12 hours
Cytotoxicity	No cytotoxicity at 200 nM concentration	
Function of signal peptide in process for protein activation	Necessary	Not necessary
Cell selectivity	Signal only through BMP receptors	Permeable all kinds of cells without help from receptors
Medical potency		Similar
Administration mode		Same

The protein described in the cited '649 patent was obtained by animal cell culture. While it is understood that "[a] composition of matter is not limited by the process by which that composition is made..." (Advisory Action, p. 2.), the method of manufacture is noted in order to distinguish the resulting protein from the claimed polypeptide. By the method of the '649 patent a protein is obtained that has a 3-dimensional structure illustrated by Fig. 2 of the present invention. BMP must have such structure in order to be secreted from a cell or to bind a receptor of cell surface.

The protein described in the '649 patent is polypeptide binding BMP receptor of cell membrane and having biological activity.<sup>7</sup> Secreted proteins such as BMP2, as described in '649 patent

<sup>7</sup> *Nature Rev. Genetics* 2003, 4; 763-773; *Neurosurg. Focus* 2002, 13, 1-6; *Endocrinol. Rev.* 2004, 25, 72-101; *J. Bone Joint Surg.* 2001, 83A, S1-S6; *Dev. Biol.* 2002, 250, 231-250; *Trends in Biotechnol.* 2001, 19, 255-265; *Dev. Biol.* 2001, 239, 1-14.

maintain biological activity throughout various post-translational modification processes. However, since a method for protein production using prokaryotic organisms cannot have such posttranslation modification processes, this method has significant problems in commercial use.

Most proteins expressed from prokaryotic organisms form biologically inactive inclusion bodies.<sup>8</sup> There have been many efforts made to induce restructuring/refolding of these proteins to achieve the physical and chemical characteristics of native protein, but it is very difficult to induce such.<sup>9</sup>

The present invention, however, provides solution to both the need for a biologically active protein and the need to be able to produce such from a prokaryotic organism and therefore provides a polypeptide that may be used in commercial processes. The biologically inactive polypeptide is produced from a prokaryotic organism (*E. coli*, using 8M Urea) and is denatured/unfolded into a linear structure, in order to maximize surface energy.<sup>10</sup> This structure is shown in the application at Example 7 and in Figs. 21-23, 28, and 29. Since polypeptides of the present invention are denatured by 8M urea as described in the detailed description and the examples, they have no original 3-dimensional structure and therefore no biological activity and do not bind the BMP receptor of cell membrane. As is seen in Figs. 21-23, 28 and 29, polypeptides according to the present invention are mostly transduced into the cells within one hour. Such characteristics are caused by high surface energy of non-structural denatured protein and PTD.

Accordingly, the TRP recited by the claims has completely different physical and chemical characteristics from the protein of the '649 patent, as demonstrated by the above different characteristics and the showing of long felt need in the art.

Additionally, the '649 patent describes a method for producing purified BMP-2 through an intracellular process by inserting a DNA sequence into cell. Specifically, the '649 patent simply describes obtaining mature BMP protein by inserting a proBMP-encoding gene, not proBMP protein, into a cell to biosynthesize mRNA from a gene expressing BMP-2 introduced into a specific cell, transferring the mRNA to the cytoplasm, biosynthesizing a protein using the mRNA

<sup>8</sup> Marston, F.A.O. Biochem. J., 240:1, 1986) (Exhibit C); Daniel et al., *Science*, 215:5, 1982; Application Note: HiTrap Chelating; 18-1134-37 AB, 1999-09, p. 1. (Exhibit D).

<sup>9</sup> Sergei V. Krivov & Martin Karplus, PNAS, 101(41):14766, 2004 (Exhibit E).

<sup>10</sup> Branden, p. 90-95; See also world wide web address page lsbu.ac.uk/water/protein2.html(Exhibit B); Sergei V., et al.; Nagahara, H., et al., *Nat. Med.*, 4: 1449, 1998.

in ribosome and extracellularly secreting the synthesized protein through post-translational modification. In other words, the '649 patent does not suggest that extracellularly synthesized proBMP protein be directly inserted into a cell.

Furthermore, the examiner's attention is respectfully drawn to pages 18482-18483 of Leighton et al., where it is described that prodomain cleavage by a furin is important for the activation of BMP-1 in a process of activation and secretion of proBMP-1. However, Leighton also state that in the absence of prodomain cleavage, BMP-1 protein is still secreted. These descriptions, to one of skill in the art, would therefore remove the motivation to introduce proBMP polypeptide into cells, when BMP-1 is similarly secreted, in order to obtain an effect such as tissue regeneration.

Additionally, Leighton et al. disclose an intracellular biosynthesis and a post-translational modification in the trans-Golgi network, but the present invention relates to introduction of a protein having its 3-dimensional structure removed, which is synthesized in a laboratory, into a cell. Unlike the intracellular biosynthesis described in Leighton et al., prior to the present invention, the intracellular process in activating a protein introduced into a cell, such as that described in the present invention was unknown. For this reason, it cannot be said that one of skill in the art would be motivated to obtain the presently claimed polypeptide from the activation of BMP protein synthesized in the cell as disclosed in Leighton et al.

**Therefore, the hBMP proprotein is not an equivalent of the claimed FAD-TRD elements of the claimed TRP and one of skill in the art would not have been motivated to substitute proBMP protein for BMP protein in the structure of PTD-BMP protein in the '867 publication by combining the '649 patent and Leighton et al. Accordingly, the combination of references does not describe either a combination of a prodomain-containing BMP and a PTD, nor does the combination of references describe insertion of such a polypeptide into a cell.**

Furthermore, on page 4 of the Final Office Action, the examiner states that "[t]he reference [the '867 publication] discloses BMP2 fused with the PTD of TAT. Experimental data is not necessary for anticipation of the polypeptide." In addition, the examiner cites the applicants as arguing "that the '867 publication 'asserts that bone formation will be induced' (Remarks page 11, paragraph 1) but that the reference does not correctly describe the mechanisms by which PTD-BMP would achieve this result" and that "the '867 publication fails to suggest intracellular delivery of the fusion protein, but that 'the present invention concretely suggests transfer of

secreted protein BMP to cells, the processes of cleavage and activation by furin in cells and the secretion of activation protein”. In reply, the examiner “asserts that any results such as bone regeneration, intracellular delivery, and protein activity are merely inherent features of the fusion polypeptide product...”

Applicants respectfully disagree. As stated above, the functional aspect of the claimed polypeptide is an essential feature of all of the claimed polypeptides and compositions of the present application. Indeed, the activity of all polypeptides with the structure recited in claim 1 cannot be said to have the same functional activity and the functional activity recited in the claims is not inherent in all structures such as those recited in claim 1.

From the results of Example 1 in the description of the present invention, it can be seen that PTD-hBMP (as described in the ‘867 publication) has cell permeability but its insertion does not provide the effect of inducing bone formation or for secreting mature BMP-2 protein which has the ability of inducing bone formation. (Specification, p. 25, lines 24-28; page 28, lines 4-20). This result is expected in view of the fact that a secretory protein having physiological activity, such as BMP-2, needs to undergo proper cellular processes such as various post-translational modifications after it is synthesized in a cell in order to have such activity.

Therefore, the PTD-BMP of the ‘867 publication is PTD with mature BMP-2, and the present application suggests in Example 1 that such structure has no biological activity. Thus, contrary to the examiner’s statement, bone regeneration, intracellular delivery and protein activity are not “inherent features of the fusion polypeptide product.”

Additionally, it cannot be said that the ‘867 publication is predictive of the product according to the present invention. The present invention suggests that proteins can have various biological activities and permeability into a cell, depending on their secondary and tertiary structure even if the proteins have the same amino acid sequences, which is an important feature of the present invention. However, the ‘867 publication provides no prediction or description with respect to activity and/or permeability dependent on structure.

Furthermore, the examiner states that “[e]xperimental data is not necessary for anticipation of the polypeptide” regarding the ‘867 publication. Applicants respectfully disagree.

The ‘867 publication provides the following:

An increase in the expression of LMP, intracellular protein  
increase the expression of mature BMP-2, and the mature BMP-

2 is secreted extracellularly to bind with a receptor in cell membrane, thus activating SMADs signal transduction.

Based on such reasoning, the '867 publication suggests that LMP, BMPs and SMADs will be used to induce bone formation by combining with PTD. However, SMADs have a plurality of down-stream genes involved in bone formation and each down-stream gene also regulates other thousands of genes. Consequently, the examiner's statement would therefore be applicable to anticipation of the combination of thousands of genes with PTD with no requirement of experimentation.

Additionally, since the '867 publication suggests that both SMADs for inducing bone formation and other SMADs for inhibiting bone formation are used for bone-formation polypeptide technique, it cannot be said that the '867 publication renders the subject matter of the present claims obvious.

As is demonstrated in Example 2 of the present invention, the fusion protein according to the present invention is activated by furin, despite the lack of a signal peptide. This means that the fusion protein according to the present invention has different intracellular processing from BMP biosynthesized in cells to be secreted. Signal peptide plays a role of transferring the BMP synthesized in ribosome to endoplasmic reticulum (ER) and Golgi when BMP is biosynthesized in cells, and this process is essential for secreting proteins having biological activity (Sakaguchi M., Curr. Opin. Biotechnol., 8:595, 1997). However, as in Example 4 of the present invention, the fusion protein according to the present invention has higher activity in case of the absence of signal peptides. In other words, it does not need such processes.

Additionally, the proteins biosynthesized in cells have their inherent secondary and tertiary structure by various molecular interactions, in particular hydrogen bond and disulfide bond (Branden C. & Tooze J., Introduction to protein structure, 2<sup>nd</sup> Ed. 1999, Garland Publishing, USA). In addition, as stated by the examiner, because the protein such as BMP has RX(K/R)R furin cleavage motif, the cleavage of prodomain consequently occurs before BMP is secreted out of the cell. However, this intracellular processing is possible under the assumption that the protein has its own secondary and tertiary structure, which is obvious to one skilled in the art, so Leighton et al. do not describe this. However, although the polypeptide of the present invention has the same amino acid sequences as described above, it has no secondary and tertiary structure because of denaturing process by Urea. Therefore, although the polypeptide of the present invention has RX(K/R)R furin cleavage motif, it cannot be said that the processing described in



Leighton et al. would naturally occur. That is to say, since the processing as described in the Leighton et al. can be carried out only after all the processes such as intracellular permeation into cells, escape from lipid raft, exposure to water environment and restructuring of PTD-BMP, are successfully preceded. Therefore, it cannot be said that, without experimental basis, Leighton et al. provide any motivation to complete the present invention.

Therefore, since Leighton et al. just suggest the intracellular biosynthesis mechanism regarding BMP, it cannot be said that there is the motivation to derive the claimed invention.

The present inventors recognized the problems in the prior art that the clinical application of the commercially available conventional hBMPs has been limited because of extremely high cost and inconveniences due to activity loss thereof in storage, handling and administration, and undertook efforts to develop a polypeptide having a novel biochemical structure and pharmaceutical mechanism, which has low production cost, is convenient in storage, handling, and does not cause a reduction in its activity in administration steps, thus resulting in the present invention. The present invention is based on the new finding **that in the case of providing the fusion polypeptide of PTD-FAD-TRD, it is much easier to prepare, store, handle and administer such fusion polypeptide because it is no longer necessary to maintain TRD as an active structure.**

However, Leighton et al. just describe that the removal of prodomain activates proteinase (Abstract). The '649 patent just suggests the method for producing purified BMP-2. Both references relate to a method for providing active BMP and they do not have the same objective or subject matter as the present invention.

Additionally, the '867 publication provides that the bone formation will be induced when LMP, which is PDZ-LIM protein, is recombined with PTD (protein transduction domain) and then administered, based on which it suggests that when LMP-1 is overexpressed in culture cells by using adenovirus the expression of intracellular mRNA is increased, and the level of intracellular BMP-2 and BMP-7 proteins is increased ('867 publication, FIG. 2). However, the '867 publication does not show recognition and a solution regarding the problem of the inconvenience in the production, storage, handling, and administration of active BMP.

As the '867 publication in view of the '649 patent and Leighton et al. does not provide any logical basis for the polypeptide or composition recited in claims 1-9 and 18-28, the '867 publication in view of the '649 patent and Leighton et al. does not render the claimed invention

obvious. Accordingly, withdrawal of the rejection of claims 1-9 and 18-28 under 35 U.S.C. § 103 (a) as being obvious over the '867 publication in view of the '649 patent and Leighton et al. is respectfully requested.

#### **Fees Payable**

The following fees due are being paid by on-line credit card payment at the time of EFS submission of this Response.

By the present Amendment, 1 new claim has been introduced, beyond the numbers for which payment was previously made. The fee is **\$26.00** for such added claim.

Additionally, a three month extension of time is requested with the present Response. The fee for such extension for a small entity is \$555.00, as specified in 37 C.F.R. § 1.17(a)(3). A one month extension of time fee in the amount of \$60.00 was paid with the Response filed on August 11, 2008. Therefore, **\$495.00** is submitted for the balance of the extension fee.

Also submitted is the fee of **\$405.00** for the RCE.

The total fee being submitted by on-line credit card payment is **\$926.00**. Should any additional fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

#### **CONCLUSION**

Based on the foregoing, all of Applicants' pending claims 1-9 and 18-28 are patentably distinguished over the art, and are in form and condition for allowance. The Examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the April 10, 2008 Office Action without extension was set at three months, or July 10, 2008. Applicants hereby request a three (3) month extension of time under 37 C.F.R. § 1.136 to extend the deadline for response to and including October 10, 2008. The fee for such extension is submitted as discussed above. Additionally, an RCE is provided with the present Response.

If any issues require further resolution, the Examiner is requested to contact either of the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

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Enclosures:  
Exhibits A-E  
RCE Transmittal

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